

Toxicity of Inhaled Methyl Isocyanate in F344/N Rats and B6C3F1 Mice. I. Acute Exposure and Recovery Studies

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Male and female F344/N rats and B6C3F1 mice were exposed to lethal and sublethal concentrations of methyl isocyanate by inhalation. Mortality, clinical signs, body and organ weights, and changes in clinical pathology and hematology were monitored immediately after 2-hr exposures and during the ensuing 3 months. Additional studies investigated the possible involvement of cyanide in the toxicity of methyl isocyanate. During exposures, signs of restlessness, lacrimation, and a reddish discharge from the nose and mouth were evident in rats and mice. Following exposures, rats and mice were dyspneic and weak. Deaths of rats and mice exposed to lethal concentrations (20 to 30 ppm) began within 15–18 hr, with males more prone to early death than females. A second wave of deaths occurred after 8 to 10 days, affecting primarily female rats and mice exposed to 20 to 30 ppm of methyl isocyanate, and male and female rats exposed to 10 ppm. Most deaths occurred during the first month following the exposures and were preceded by periods of severe respiratory distress. Body weights decreased in proportion to dose early, but then weight gain resumed in survivors at control rates. The only organ with a consistent, dose-related weight change was the lung, which was heavier throughout the studies in animals exposed to high concentrations of methyl isocyanate. No significant clinical pathology, or hematologic changes were observed in exposed rats. Blood and brain cholinesterase were not inhibited. Studies attempting to measure cyanide in the blood of methyl isocyanate-exposed rats, and attempting to affect lethality with a cyanide antidote (sodium nitrite and sodium thiosulfate) gave negative results. The findings indicate that at these doses, methyl isocyanate inhalation causes deaths and persistent pulmonary changes, but no evidence of extrapulmonary toxicity in rodents. Cyanide does not appear to be involved in methyl isocyanate toxicity.

Introduction

Shortly after midnight on December 3, 1984, a toxic gas was released from an agricultural chemical plant in Bhopal, India. Approximately 50,000 lb of material escaped into the atmosphere from an overheated tank used to store methyl isocyanate (MIC). A foglike cloud spread over an approximate 60 km² area, exposing approximately 200,000 people. Estimates of deaths and injuries vary, but the Indian Government numbered fatalities at about 2000, with tens of thousands seriously injured (1,2).

Shortly after the accident, the World Health Organization requested that the National Toxicology Program (NTP) perform studies to examine the long-term health effects of exposure to methyl isocyanate. In response to this request, the NTP initiated a series of studies to assess effects of MIC inhalation on the respiratory system, to determine the potential for systemic

toxicity, including specific assessments of immune and reproductive function, and to examine the potential for genetic toxicity. This report details several aspects of the toxicity of inhaled methyl isocyanate in male and female F344/N rats and B6C3F1 mice. Mortality, clinical signs, and changes in clinical pathology, hematology, and body and organ weights were determined immediately after 2-hr inhalation exposures, and during the ensuing 3 months. In addition, the efficacy of a cyanide antidote (sodium nitrite, sodium thiosulfate) to affect MIC toxicity was evaluated because of anecdotal reports of significant improvement in exposed people given sodium thiosulfate (3). Results of histopathologic analyses, and studies of immune, reproductive, and genetic toxicity are reported separately.

Materials and Methods

Chemical

Methyl isocyanate was supplied by Union Carbide Corporation. The chemical was obtained in stainless

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steel cylinders. Chemical analysis by the manufacturer indicated the following composition: MIC, 99.65%; chloroform, 0.02%; MIC dimer, 0.27%; 1,3-dimethylurea, 0.01%; MIC trimer, 0.01%; 1,3,5-trimethylbiuret, 0.02%; phosgene, 0.02%. NTP analysis (GC/MS) confirmed the greater than 99% purity of the material.

Exposure Conditions

Exposures were carried out in 1330-L, stainless steel chambers. Chamber atmospheres were generated through a two-stage dilution system, with dry nitrogen as a carrier gas. Two analytical methods were used to monitor MIC concentrations in the exposure chambers, in the chamber room, and in the scrubbed effluent. Chamber concentrations were monitored continuously at 3.3 μ m with a Wilks Miran-80 IR Spectrometer. Control chamber, room, and effluent gases were analyzed according to the method of Vincent and Ketcham (4). This technique involves collection of air samples on XAD-2 resin, followed by elution with tetrahydrofuran and HPLC analysis of eluted material after reaction with fluorecamine. Chambers were operated with flows of from 300 to 500 L/min, at 20–26°C and 40–60% relative humidity. Complete details of generation, monitoring, and safety aspects are reported in an accompanying paper (5). Exposures were conducted on 3/27, 4/22, 5/30, and 6/5/85.

Animals

Male and female F344/N rats (4–6 weeks old) and male and female B6C3F1 mice (4–8 weeks old) were obtained from Charles River (Kingston, NY, or Portage, MI). Animals were quarantined for 10 to 21 days prior to exposure. Animals were randomized to exposure groups according to body weight, and were housed 4 (rats), or 5 (mice) per cage, except during exposures, when mice were housed 10 per cage. Food (autoclaved NIH-31, Zeigler Bros., Gardners, PA) and water were available *ad libitum* except during exposures.

Experimental Procedures

Rats and mice were exposed to concentrations of MIC from 0 to 30 ppm for 2 hr. Within 3 hr following the exposures (day 0), and again on days 1, 3, 7, 14, 49 and 91 after exposure, five predesignated animals per group were killed by pentobarbital overdose and given a complete gross necropsy. The lungs, brain, liver, kidney, thymus, testis, and spleen were weighed prior to fixation for histopathologic analysis [see accompanying papers (6,7)]. The right apical lobe of the lung was removed from male rats killed on days 0, 1, 3 and 7, weighed, and allowed to dry to constant weight for determination of wet-to-dry weight ratio. Immediately prior to necropsy, blood was collected from the right cardiac ventricle of anesthetized male rats on day 0, 1, and 7 and from female rats on days 1, 7, and 14. Each

sample was divided, one fraction was allowed to clot for serum collection, and the other was added to a tube containing a premeasured amount of EDTA. Serum samples were analyzed for activities of alanine aminotransferase (8), alkaline phosphatase (9), creatine kinase (10), and sorbitol dehydrogenase (11), and concentrations of urea nitrogen (12) and creatinine (13). Samples of whole blood were used for determinations of total blood cell counts (laser optics counter), for preparation of blood smears (morphologic evaluation and differential counts) and for measurements of concentrations of methemoglobin (14) and activities of cholinesterase (15). Cholinesterase activity was also determined in homogenates of brain tissue. For these analyses, the right hemisphere was taken, chilled, and homogenized in 10 volumes of 0.25 M sucrose. Animals were checked for mortality, morbidity, and clinical signs twice daily throughout the first month, and once daily thereafter.

Viral Serology and Sentinel Animal Program

Upon arrival, five to ten rats and mice from each shipment were cultured and serologically tested for microbial pathogens using methods described by Thigpen and Ross (16). Rats tested negative for antibodies to RCV/SDA, Sendai, KRV, PVM, and H-1. Mice tested negative for antibodies to MHV, Sendai, PVM, GDVII and EDIM. During 91-day studies, pathogen-free F344/N rats and Cr1:COBS CD (SD)1 mice were placed in animals rooms, and were taken for analysis at monthly intervals. In addition to serological screening, fecal specimens were examined for parasites and cultured for enteric pathogens, including *Salmonella* sp. Lungs and nasal washes were cultured for respiratory pathogens including mycoplasma sp. (16). All sentinel animals were culture negative for all pathogens tested, with the exception that *Hexamastix* sp., a flagellated protozoan, was present in most of the rats and mice.

Cyanide Antidote Studies

The blood of male rats exposed to MIC was collected within 1 hr, and at 48 hr following exposure for CN analysis. Blood was obtained from the heart and analyzed for CN by a modification of the method of Feldstein and Klendzkoj (17). The minimum quantitative level of this modified assay is 50 ng of CN/g of blood.

In other studies, male rats were given single or multiple doses of sodium nitrite (25 mg/kg, SC) and sodium thiosulfate (250 mg/kg, IP), by injection, 1 hr prior to, and twice weekly, following 2-hr exposures to various concentrations of MIC. Mortality was then monitored for 45 days.

Statistical Methods

Statistical analyses for survival differences used the method of Cox (18) for testing two groups for equality.

Other data were analyzed using the RS/1 Multicompare procedure, using the Wilk-Shapiro test for normality. The unpooled variance *t* test was used to test for differences in body and organ weights, clinical pathology and hematology. Data expressed in the form of ratios (organ to body weight, wet to dry weight) were analyzed using the Wilcoxon rank sum test.

Results

Mortality and Clinical Signal

During exposures, rats initially were irritated and restless at concentrations of 10 to 15 ppm. Grooming increased, and rubbing of eyes and ears was noted. At 20 ppm, rats walked with a low carriage with heads down. Eyes were kept partially closed. As concentrations approached and exceeded 30 ppm, rats often lay flat on their bellies, with eyes closed. Lacrimation was excessive, and a frothy, often reddish discharge was noted from the nose and mouth. Rats were unresponsive to noise, but remained conscious. Mice also showed a lessening of activity at high concentrations of MIC, but clinical signs of distress were not as apparent as in rats, and no discharge from the nose or mouth was seen. Many mice appeared to sleep through the exposures.

No animals died during the 2-hr exposures. Deaths of mice were observed within 15 to 18 hr following exposures to 30 ppm (Fig. 1), and rats began to die within a similar period after exposures to as low as 20 ppm (Fig. 2). Gross examination of animals dying during this early period (days 1–3) revealed profuse edema and exudate in the nasal cavity and upper airways, and extending into the trachea and large bronchi.

Although mortality varied from one exposure to the next, several general patterns were consistent between experiments. A higher proportion of male rats and mice than females died during the first 1 to 3 days following

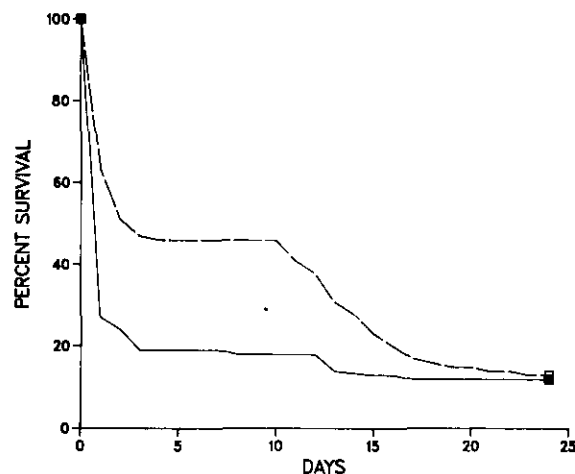


FIGURE 1. Survival of (—) male and (---) female mice following exposure to 30 ppm MIC (29.96 ± 3.31 SD), for 2 hr, $n = 100$ per exposure group.

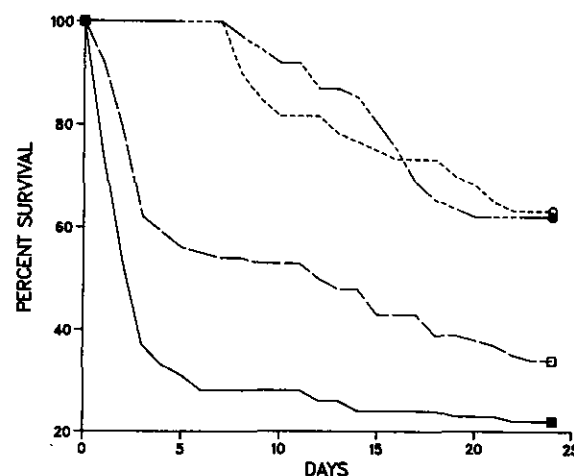


FIGURE 2. Survival of (●) male and (○) female rats following exposure to (□) 10 (8.88 ± 0.48 SD), or 20 ppm females (19.27 ± 0.78 SD), or 20 ppm (males, 18.95 ± 0.63) MIC for 2 hr; $n = 100$ for 20 ppm, and $n = 60$ for 10 ppm.

exposure to 20 or 30 ppm, and females in particular showed a biphasic survival curve. Initial deaths were followed by a 5- to 7-day period in which few deaths occurred. Deaths then resumed, and cumulative mortality was similar between the sexes after about 3 weeks. This delay in onset of mortality was also seen in male and female rats exposed to 10 ppm MIC for 2 hr (Fig. 2). Deaths began on day 8, and overall survival of male and female rats exposed to 10 ppm for 2 hr was similar. No mice died after exposure to 10 ppm. Most deaths of exposed animals occurred during the first month after the exposures, and were preceded by periods of severe respiratory distress. The last deaths occurred on days 49 and 78 after exposure. These were both male mice exposed to 30 ppm. No deaths of control animals occurred during the studies.

Upon removal from the chambers, rats showed dose-related clinical signs which included weakness, ruffled haircoat, and respiratory distress characterized by gasping, moist rales, open mouth, and abdominal breathing. Breathing in rats and mice was often accompanied by audible snaps and wheezes. Male rats and mice subjectively appeared more affected than females. Clinical signs persisted in survivors, but lessened in intensity through about day 7 after exposure. Signs of respiratory distress then worsened especially in females, coincident with the resumption of deaths. Affected rats often sat on haunches or clung to cagemates in an effort to maintain a vertical orientation. Mice had similar but less discernable signs of dyspnea that coincided with periods of high mortality. Deaths of rats and mice were occasionally abrupt and preceded by periods of hyperactivity. Eating and drinking lessened during periods of respiratory distress. Evidence of dyspnea then moderated in survivors and was no longer evident beyond day 28.

Body and Organ Weights

As shown in Figure 3, body weight gains showed dose-related patterns with high dose animals initially losing weight and the survivors then generally gaining weight at a rate equal to that of controls. The minimum weight of the 30 ppm-exposed female rats occurred later than those of the other high dose groups, but coincided with the maximum period of respiratory distress and mortality in high dose females in this experiment. All male rats exposed to 30 ppm died within 28 days following the exposure.

Organ weights collected during these experiments included brain, lung, liver, kidney, thymus, spleen, and testis. Changes in absolute organ weights and in organ-

to-body weight ratios for brain, liver, kidney, spleen, and testis (not shown) were statistically significant at certain time points in certain experimental groups, but the differences were not consistent between groups, and did not suggest a selective toxicity to any of these organs. With the exception of mild cytoplasmic vacuolization of the liver in mice, significant pathologic changes were not found in these organs (6,7). However, changes in lung- and thymus-to-body weight ratios were consistent and were dose-related in mice and rats. Thymus-to-body weight ratios fell 50 to 70% from initial values in 30 ppm male and female mice and male rats within 3 days following exposure (not shown). The minimum ratio in female rats occurred at day 14 and coincided with the minimum body weight of these animals. The his-

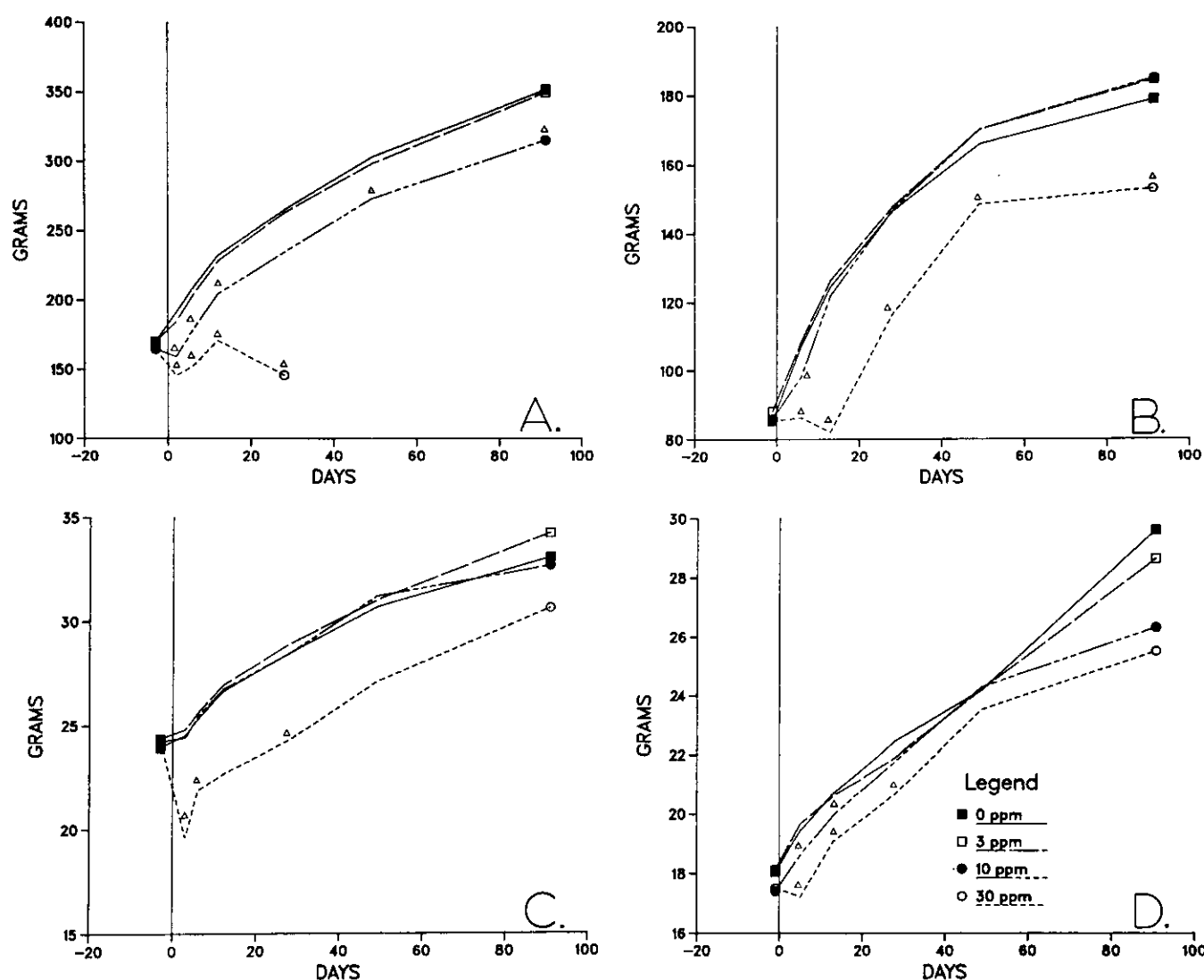


FIGURE 3. Mean body weights of (A) male rats, (B) female rats, (C) male mice, or (D) female mice, following exposures to 0, 3, 10, or 30 ppm MIC, for 2 hr; $n=40$ for initial time points, and n decreased by 5 on each analysis day (see "Materials and Methods") (Δ , $p<0.05$). For high dose groups experiencing mortality the n decreased more rapidly, but was at least 5, except for male rats at day 28 ($n=4$), and female rats at day 91 ($n=2$). Average chamber concentrations were: male rats and mice, 3.12 ± 0.53 SD, 9.23 ± 1.46 , 28.84 ± 1.59 , female rats and mice, 3.03 ± 0.28 , 9.76 ± 0.46 , 30.07 ± 0.51 .

tologic appearance was characterized as atrophic during this time; however, thymic weight and microscopic appearance did not differ from controls after day 28 in all groups, suggesting effects on the thymus were secondary to the general health of the animals, and were not an indication of a selective toxicity or exposure to the thymus.

Significant changes in lung weights and lung-to-body weight ratios were seen in exposed animals of both sexes and species. An immediate (day 0), dose-related increase in lung weight was seen in male rats (Fig. 4A), the group most susceptible to early death following exposure. Lung wet-to-dry weight ratios suggested a dose-related increase immediately following the exposure, but the changes were not statistically significant (0 ppm, 4.35 ± 0.10 ; 3 ppm, 4.28 ± 0.17 ; 10 ppm, 4.54 ± 0.19 ; 30 ppm, 4.62 ± 0.31). Wet-to-dry weight ra-

tios were not consistently elevated in dosed male rats on days 1, 3, or 7 after exposure. However, lung weights increased dramatically in male rats in the 10 and 30 ppm exposure groups, and were significantly elevated in 10 ppm animals on day 91; all 30 ppm male rats died by day 28. Female rats exposed to 30 ppm did not show an immediate increase in lung weight, and this is consistent with the less intense early signs of respiratory distress in females than males (Fig. 4B). However, these animals showed a consistently heavier lung by day 14 after exposure and beyond, and this was associated with significant delayed mortality in this particular experiment. High dose male mice showed increased in lung weight as early as day 1 (Fig. 4C), but statistical significance was not reached until day 3. Lung-to-body weight ratios were significantly greater in 30 ppm mice than in controls at all scheduled sacrifice times including

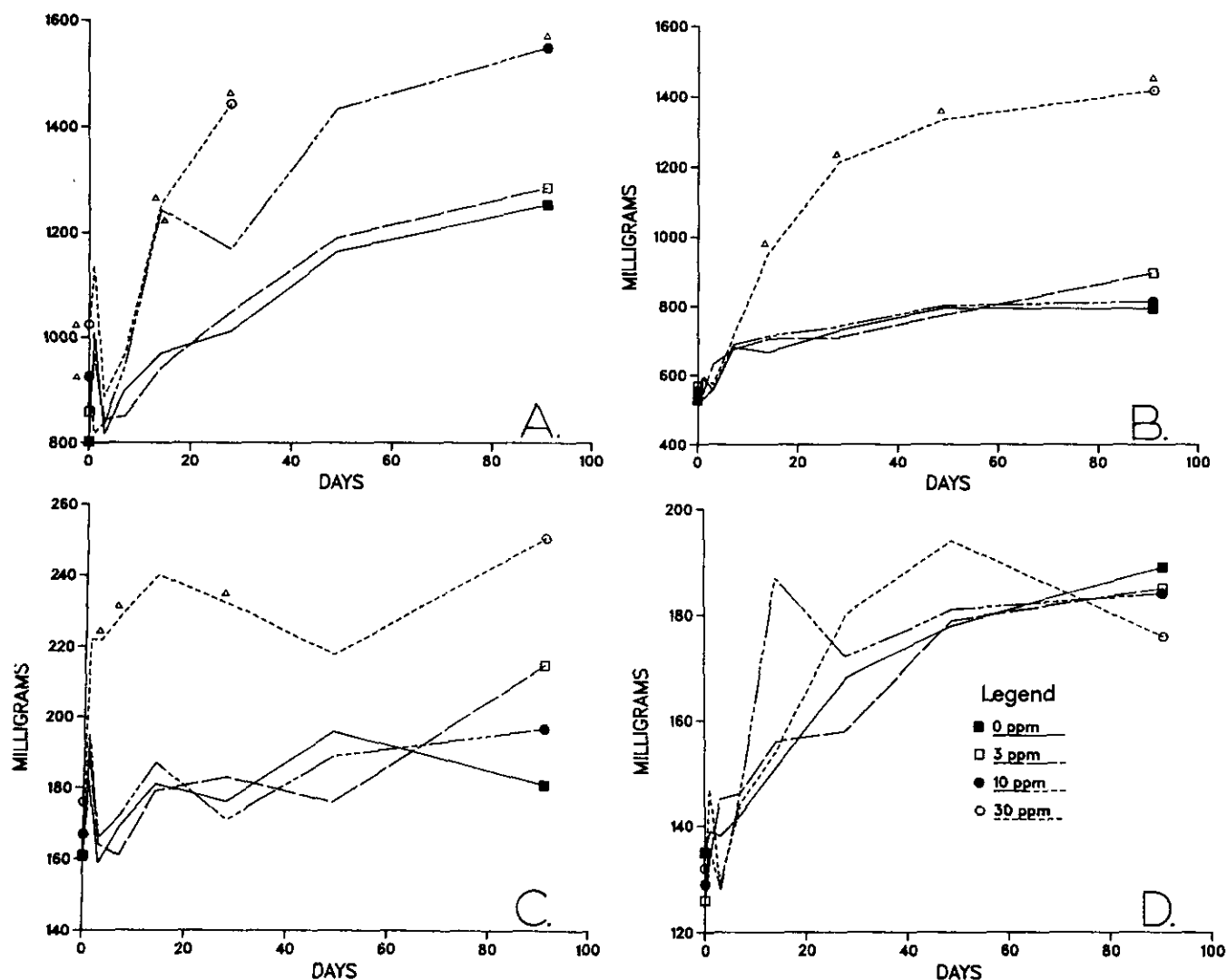


FIGURE 4. Lung weights of (A) male rats, (B) female rats, (C) male mice, and (D) female mice following exposures to 0, 3, 10, or 30 ppm MIC, for 2 hr, $n=5$, except for male rats at day 28 ($n=4$), and female rats at day 91 ($n=2$); (Δ , $p<0.05$). For exposure concentrations see Fig. 3.

day 91 (not shown). Increases in female mice lung weights were not seen following exposure to methyl isocyanate.

Clinical Biochemistry and Hematology

A significant increase ($p < 0.05$) in the serum activity of alanine aminotransferase occurred only in the high dose group of female rats at day 14 after exposure (Table 1). This change was mild (approximately 2 times) and was accompanied by a similar but insignificant increase in sorbitol dehydrogenase. Although these enzymes are specific for hepatocellular damage (leakage or necrosis), no overt necrosis was seen histologically, and the mild changes were consistent with secondary exposure effects related to dehydration and decreased hepatic perfusion. Serum activities of alkaline phosphatase decreased in high dose male and female rats at all time points except in males on day 0. These changes were significant in the male rats at 1 and 7 days and in the female rats at 14 days. Unrelated to biliary lesions, activities of alkaline phosphatase can increase with feeding and conversely, decrease during periods of fasting. In this study, the decreased activities were observed in animals in those groups that had decreased body weights and moderate to marked clinical signs. The decreases are consistent with those produced by decreased food intake. No treatment-related effects were detected in concentrations of blood urea nitrogen, creatinine, and methemoglobin or in activities of cholinesterase in whole blood or brain. Serum creatine kinase showed dose-related increases in male rats on days 0 and 1 and in female rats on days 1, 7, and 14, but these increases were mild and not statistically significant (not shown).

Total white cell counts decreased (not significant) in all exposure groups of male rats on day 0, and in the 10 and 30 ppm groups ($p < 0.05$) on day 1. Changes in total white cell counts did not occur at other times in the remaining groups of male or female rats. Segmented neutrophils increased in the high dose and occasionally in the middle dose groups of male and female rats at all sampling times with the exception of day 1 for male rats (Table 2). Additionally, lymphocyte counts decreased in male rats exposed to 3, 10, and 30 ppm on day 0, to 10

and 30 ppm on day 1, and to 30 ppm on day 7. In female rats, decreases in lymphocytes were mild and confined to rats in high dose groups at 1 and 14 days. These changes (mild to moderate increase in mature neutrophils and an accompanying moderate decrease in lymphocytes) are consistent with a stress leukogram resulting from the release of endogenous corticosteroids. There were no alterations in absolute counts of immature neutrophils, eosinophils, monocytes, or basophils. Platelet counts in exposed animals were significantly different from controls in some groups, but the changes were mild and random and not considered biologically significant (not shown).

With the exception of counts in the male rats at day 0, red cell counts increased significantly in rats in all high dose and several lower dose groups (Table 3). The increased counts were accompanied by similar increases in hematocrit and hemoglobin concentrations. These changes are consistent with effects of dehydration and hemoconcentration. Red cell indices (mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration) were similar to controls in all exposure groups.

Cyanide Antidote Studies

For these studies, groups of 30 male rats were given no antidote, or were given one injection of sodium nitrite (25 mg/kg), and sodium thiosulfate (250 mg/kg) 1 hr prior to the exposure to MIC; one group was given additional doses of antidote two times per week for the duration of the studies. These doses were chosen based on results of studies which examined the toxicity of the antidote, and the efficacy of the antidote to prevent deaths of rats given potassium cyanide.

In preliminary studies, male rats given 50 mg/kg sodium nitrite and 500 mg/kg sodium thiosulfate and not exposed to MIC showed signs of cyanosis and reduced activity within 30 min of dosing, and 1/20 rats died. Rats given 25 mg/kg of sodium nitrite and 250 mg/kg sodium thiosulfate did not show signs of cyanosis or other evidence of toxicity. When rats given this level of antidote were challenged with 15 mg/kg potassium cyanide by

Table 1. Clinical pathology in male and female rats exposed to methyl isocyanate.

MIC exposure, ppm	Alanine aminotransferase (ALT) U/L \pm SD				Sorbitol dehydrogenase (SDH) U/L \pm SD			
	0	1 day	7 days	14 days	0	1 day	7 days	14 days
Males								
0	36 \pm 3	46 \pm 7	59 \pm 11		7 \pm 2	7 \pm 2	10 \pm 2	
3	37 \pm 3	40 \pm 2	54 \pm 3		9 \pm 2	7 \pm 1	10 \pm 2	
10	38 \pm 4	40 \pm 4	53 \pm 13		7 \pm 1	10 \pm 3	9 \pm 1	
30	42 \pm 7	50 \pm 9	46 \pm 10		8 \pm 2	15 \pm 6	8 \pm 2	
Females								
0		42 \pm 4	37 \pm 5	34 \pm 5		6 \pm 2	7 \pm 0	7 \pm 1
3		40 \pm 6	35 \pm 2	30 \pm 2		7 \pm 2	7 \pm 2	5 \pm 0*
10		27 \pm 3	37 \pm 4	32 \pm 2		6 \pm 1	7 \pm 4	7 \pm 2
30		35 \pm 6	41 \pm 9	79 \pm 40*		7 \pm 2	14 \pm 12	16 \pm 9

* $p < 0.05$.

Table 2. Segmented neutrophil and lymphocyte counts in male and female rats exposed to methyl isocyanate.

MIC exposure, ppm	Segmented neutrophils, 1000/mm ³ ± SD				Lymphocytes, 1000/mm ³ ± SD			
	0	1 day	7 days	14 days	0	1 day	7 days	14 days
Males								
0	0.99 ± 0.26	0.85 ± 0.25	0.83 ± 0.25		2.35 ± 0.13	2.90 ± 0.27	3.37 ± 0.28	
3	0.95 ± 0.16	0.88 ± 0.20	0.89 ± 0.18		1.77 ± 0.17*	2.96 ± 0.25	3.46 ± 0.21	
10	0.99 ± 0.24*	1.04 ± 0.24	1.59 ± 0.63*		1.50 ± 0.22*	1.67 ± 0.24*	3.52 ± 0.61	
30	1.35 ± 0.23*	0.63 ± 0.11	1.74 ± 0.13*		1.29 ± 0.27*	1.46 ± 0.13*	2.34 ± 0.14*	
Females								
0		0.54 ± 0.06	0.91 ± 0.15	0.79 ± 0.21		1.81 ± 0.11	2.88 ± 0.12	2.57 ± 0.18
3		0.96 ± 0.80*	0.87 ± 0.21	0.89 ± 0.21		2.46 ± 0.32*	2.70 ± 0.29	2.37 ± 0.28
10		0.69 ± 0.12*	1.02 ± 0.23	0.67 ± 0.06		1.75 ± 0.09	2.86 ± 0.24	2.37 ± 0.13
30		0.73 ± 0.29	1.63 ± 0.40*	1.31 ± 0.17*		1.57 ± 0.24	2.80 ± 0.40	2.22 ± 0.30

**p* < 0.05.

Table 3. Red blood cell counts in male and female rats exposed to methyl isocyanate.

MIC exposure, ppm	Red blood cells (1,000,000/mm ³ ± SD)			
	0	1 day	7 days	14 days
Males				
0	7.65 ± 0.21	7.46 ± 0.36	7.92 ± 0.18	
3	7.59 ± 0.24	7.87 ± 0.23*	7.79 ± 0.37	
10	7.54 ± 0.24	8.60 ± 0.27*	7.94 ± 0.16	
30	7.98 ± 0.30	8.57 ± 0.17*	8.93 ± 0.51*	
Females				
0		7.09 ± 0.23	7.55 ± 0.05	7.70 ± 0.21
3		7.28 ± 0.19	7.41 ± 0.26	7.32 ± 0.10
10		8.16 ± 0.22*	7.57 ± 0.26	7.60 ± 0.22
30		8.34 ± 0.48*	8.12 ± 0.58*	9.64 ± 0.83*

**p* < 0.05.

gavage, 1 hr later, 4/5 animals survived, whereas this challenge killed 5/5 control rats within 5 min.

In studies of the effectiveness of the CN antidote to prevent deaths due to MIC exposure, male rats dosed with antidote as outlined above were exposed to 0, 30, or 60 ppm MIC for 2 hr. As in other studies, no rats died in the chambers, but deaths of animals exposed to 60 ppm began within 5 hr of removal from the chambers. As shown in Figure 5, the CN antidote did not affect the pattern of mortality following exposure to 60 ppm MIC. Data for the two groups of CN antidote treated rats are shown separately, although no rats lived long enough to receive a second dose of antidote. Figure 6 shows the mortality pattern of animals exposed to 30 ppm MIC for 2 hr. In these groups, survival of rats given single or repeated doses of antidote did not differ significantly from controls.

Analyses of blood cyanide concentrations of non-antidote-treated animals upon removal from the 0, 30, and 60 ppm MIC chambers and on day 2 postexposure gave readings below the limit of quantitation (50 ng/g blood). The minimum blood cyanide concentration following a lethal dose of KCN (6 mg/kg) to male Sprague-Dawley rats is reported to be in the range of 2.4 to 2.6 µg/g (18). To confirm the sensitivity of our CN analysis method, male F344 rats were given 2.5 mg/kg KCN by gavage (1/4 the reported LD₅₀). No animals died, but CN blood levels were determined to be 800 ng/g immedi-

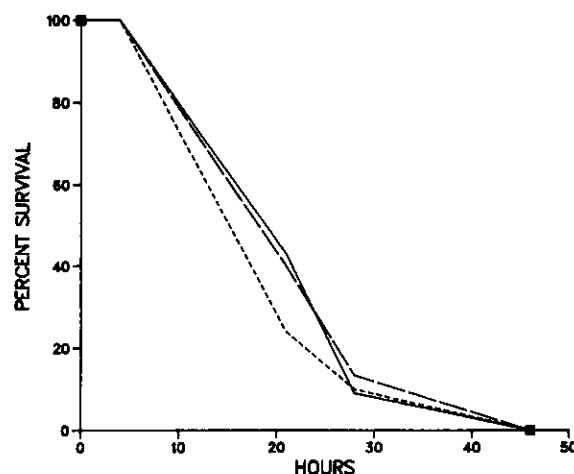


FIGURE 5. Survival of male rats (—) given no antidote or one dose of antidote (sodium nitrite, sodium thiosulfate) (two groups, broken line, and dashed line) and exposed to 60 ppm MIC (57.48 ± 1.68 SD), for 2 hr, *n* = 30.

ately after dosing. These levels declined to below the quantitation limit within 3 hr. Thus, using an assay capable of detecting sublethal concentrations of CN in the blood, we could not detect any CN in the blood of rats exposed to lethal concentrations of MIC either immediately, or 2 days after the exposure.

Discussion

Kimmerle and Eben (20) previously demonstrated the acute toxic and lethal effects of MIC vapors in rats, mice, rabbits, and guinea pigs. They observed deaths of mice during a 30-min exposure to 4 g of MIC evaporated in a 400-L chamber; deaths of rats occurred after removal from the chamber. Exposure to lower concentrations resulted in patterns of mortality not unlike those observed in rats and mice in the present studies. The majority of deaths in the earlier studies were observed soon after exposures, and deaths occurred up to 18 days later. The clinical signs of restlessness, eye irritation, and respiratory distress during exposures re-

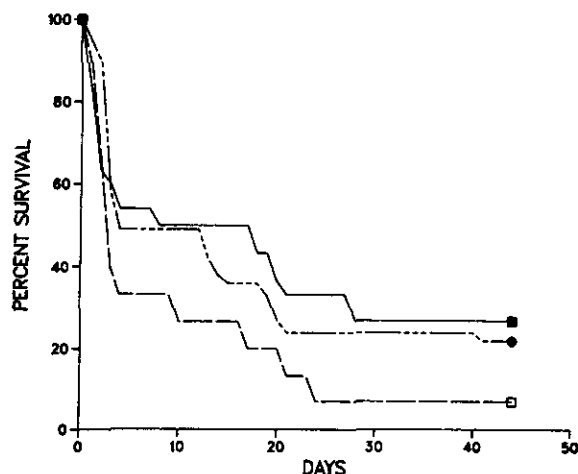


FIGURE 6. Survival of male rats (■) given no antidote, or given antidote (●) one dose prior to exposure, or (□) one dose prior to, plus twice weekly doses following, and exposed to 30 ppm MIC (29.96 ± 2.49 SD), for 2 hr, $n = 30$.

ported by Kimmerle and Eben were similar to those observed in the present studies.

We observed deaths of rats following exposures to ≥ 10 ppm and of mice exposed to 30 ppm for 2 hr. Mortality was variable between replicate exposures, but the data suggested a steep dose response, and an approximate 2-hr LC_{50} in the range of 20 to 25 ppm for rats, and somewhat higher, perhaps 25 to 30 ppm, for mice. Male rats and mice appeared more susceptible than females to death during the first 3 days after exposures. This coincided with clinical signs of severe respiratory distress and increased lung weight, suggesting the presence of pulmonary edema. Lung wet-to-dry weight ratios were higher in MIC-exposed rats than in controls immediately following the exposures. Wet-to-dry weight determinations were not done in female rats, or in mice.

A second wave of deaths was observed after 8 to 10 days, especially in female rats and mice, and in males rats exposed to 10 ppm MIC. This phase also coincided with clinical signs of severe respiratory distress, and increases in lung weight. The persistence of the increase in lung weight in animals that survived exposures to high doses of MIC suggests a proliferative response, perhaps associated with a reparative process. Microscopic examination showed peribronchiolar and intraluminal fibrosis. This, coupled with an overall increase in lung size, which was apparent upon inflation, could account for the increased lung weights. The fibrotic response was seen in both sexes. It is not clear why males and females show apparent differences in susceptibility to early death, but this may be related to a slightly larger respiratory surface area relative to body weight in male animals.

Several high dose mice died during the later part of the 91-day study. These deaths could not be associated unequivocally with prior exposure to MIC, but no spontaneous deaths occurred at any time during the studies

in control or lower dose groups. These animals were not noted to be suffering from respiratory distress immediately before death, but appeared debilitated and had lost weight.

Organ weight data suggested only secondary effects, and no specific toxicity of inhaled MIC to any nonrespiratory organ. Results of hematology and clinical pathology assessments indicated mild to moderate changes in total and differential leukocyte count, red cell count, hemoglobin concentration, hematocrit, and activity of alanine aminotransferase, sorbitol dehydrogenase, and alkaline phosphatase. MIC exposures did not affect other analyses, including activities of cholinesterase in blood and brain, and concentrations of methemoglobin. All changes were nonspecific and considered to be secondary to decreased food intake, dehydration and stress. Changes occurred more frequently in male than in female rats, which was consistent with other findings.

Increases in red cell (RBC) count, hemoglobin, and hemoglobin concentrations appeared due to hemoconcentration in animals which appeared dehydrated. However, water consumption was not monitored in these studies. It is possible that increased RBC counts could be a response to hypoxia, but the absence of significant changes in RBC indices did not suggest an influx of reticulocytes. Isolated increases in the number of nucleated RBCs were seen in 30 ppm male rats on day 1 and 30 ppm female rats on day 14.

Methyl isocyanate is a highly reactive chemical, capable of forming a variety of reaction products with water, and cellular and extracellular constituents (21–25). The reaction with water is exothermic and yields carbon dioxide and several ureas and biurets, including 1,3-dimethylurea, and 1,3,5-trimethylbiuret. MIC will carbamoylate amines, compounds containing hydroxyl groups, and sulfhydryls (21). It causes severe burns and necrosis when applied to the skin of rabbits (22). From the data presented, it would appear that the extreme reactivity of the chemical caused injury to be localized to the point of initial contact, which in these exposures was primarily the respiratory tract. No evidence of skin irritation was seen, and copious lacrimation appeared to prevent significant eye injury (7,26). No evidence of interaction with blood constituents was found. Although we did not look for evidence of carbamoylated hemoglobin (23,24), we did not observe hemolysis, nor did we see inhibition of blood cholinesterase, which theoretically could have occurred through direct carbamoylation by MIC, or through inhibition by carbamate reaction products (21).

MIC would appear to be the agent responsible for the observed toxicities. Most conceivable reaction products of MIC and cellular constituents are certainly less reactive, and likely less acutely toxic. We have found no evidence for involvement of cyanide as a cause of death either in the initial intoxication, or in subsequent days following the exposures. This does not support postulated cyanogenic pathways proposed to account for improvement in the survivors of the Bhopal accident fol-

lowing treatment with sodium thiosulfate. If sodium thiosulfate is in fact effective in lessening reported symptoms of respiratory problems and weakness (3), then it may be affecting other processes not involved in the metabolism of cyanide; alternatively perhaps cyanide was released along with MIC and other reaction products during the accident in Bhopal. Another possibility is that cyanogenic pathways, if they exist (27), may become significant only at times later than we examined, or may lead to generation of cyanide in the lung and this is not reflected in changes in blood cyanide levels.

The results of our studies suggest that no significant systemic, extrapulmonary exposure to MIC occurred during this exposure regimen. However, Salmon et al. (28), in a recent report of preliminary acute study results in which rats were exposed to concentrations of MIC from 11 to 65 ppm for 2 hr emphasized an observed sedative or "narcotic" effect of MIC on animal behavior during the exposures, and implied a direct CNS effect of MIC or a reaction product. The descriptions of clinical signs during the exposures did not differ markedly from what we observed, but the signs of sedation were less apparent in our studies, and we could not conclude that they were evidence of a CNS effect, as opposed to lessened activity and shallow breathing associated with attempts to minimize chemical exposure.

In summary, exposures of rats and mice to methyl isocyanate by inhalation were found to result in deaths, and in persistent pulmonary changes in survivors. No evidence of systemic exposure, or evidence of specific toxicity to any nonrespiratory organ was found in measurements of organ weights, hematologic or clinical pathology assessments. Cyanide does not appear to be involved in the acute toxicity of methyl isocyanate.

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